High Levels of Epstein-Barr Virus DNA in Saliva and Peripheral Blood from Ugandan Mother-Child Pairs

Sam M. Mbulaiteye,[1] Michael Walters,[2] Eric A. Engels,[1] Paul M. Bakaki,[3] Christopher M. Ndugwa,[3] Anchilla M. Owor,[3] James J. Goedert,[1] Denise Whitby,[2] and Robert J. Biggar[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Department of Health and Human Services, Rockville, and [2]Viral Epidemiology Section, AIDS Vaccine Program, Science Applications International Corporation–Frederick, National Cancer Institute at Frederick, Frederick, Maryland; [3]Makerere University Medical School and Mulago Hospital, Kampala, Uganda

In Africa, Epstein-Barr virus (EBV) is associated with Burkitt lymphoma. We measured levels of EBV DNA in saliva and buffy coats from 233 asymptomatic Ugandan children with sickle cell disease and their mothers by quantitative real-time polymerase chain reaction. EBV DNA was detected in saliva from 90% of the children (median [interquartile range {IQR}] level, 5.2 [4.2–6.0] $\log_{10}$ copies/mL of saliva) and 79% of the mothers (median [IQR] level, 4.8 [3.7–5.6] $\log_{10}$ copies/mL of saliva) ($P < .001$). EBV DNA was detected in buffy coats from 86% of the children (median [IQR] level, 2.5 [2.2–2.9] $\log_{10}$ copies/1×10^6 peripheral white blood cells [PWBCs]) and 72% of the mothers (median [IQR] level, 2.7 [2.4–3.1] $\log_{10}$ copies/1×10^6 PWBCs) ($P = .24$). Detection of EBV DNA in saliva was positively correlated with detection in buffy coats. EBV DNA was detected more frequently in saliva and buffy coats than was human herpesvirus 8 DNA. Our results indicate that EBV infection persists, with virus readily detectable in saliva and buffy coats from persons without apparent symptoms in Africa.

Epstein-Barr virus (EBV) is ubiquitous, but the age at infection varies [1]. In Africa, most infections occur during early childhood and are asymptomatic [2]; however, they are associated with endemic Burkitt lymphoma, the most common tumor in African children. In the West, infections occur mostly in older children and adolescents, who frequently present with infectious mononucleosis [1].

EBV is probably transmitted via saliva, such as when mothers prechew food that is then given to babies, when people share eating utensils, and when people kiss [1].

EBV persists in infected persons in lymphoid or epithelial cells within the oral cavity or salivary glands and intermittently reactivates, leading to viral shedding in saliva [1]. Little is known about the patterns of EBV shedding in saliva or peripheral blood in Africans. In populations in the West, where studies have quantified EBV DNA levels in saliva and peripheral blood [3–5], EBV is variably detected in healthy subjects, and the levels in individuals are typically low. Only one study has evaluated EBV levels in the peripheral blood of asymptomatic subjects in Africa; it found that EBV was readily detected at high levels in the peripheral blood of Kenyan children 1–4 years old [6]. We evaluated viral shedding in children and their mothers in Uganda, a country in which Burkitt lymphoma is endemic, to learn more about EBV prevalence, infection intensity, and shedding in Africans.

SUBJECTS, MATERIALS, AND METHODS

We studied 600 children with sickle cell disease and their mothers who, between November 2001 and April

2002, had been recruited from the Sickle Cell Clinic at Mulago Hospital, Kampala, into a study that evaluated the risk of blood-borne transmission of human herpesvirus 8 (HHV-8), a virus causally associated with Kaposi sarcoma [7]. By design, approximately one-half of the children had been transfused. The present substudy analyzed subjects who had been previously studied for HHV-8 DNA detection in rapidly frozen saliva samples and buffy coats [8], including all children who were either seropositive ($n = 143$) or indeterminate ($n = 40$) for HHV-8 by serological testing as well as 50 randomly chosen HHV-8–seronegative children. The mothers of these subjects, when available, were also studied. Because EBV infection occurs by 2 years of age among Africans [2], EBV serological testing was not performed. Permission to test for HIV infection was not obtained and, therefore, was not done. No subject was known or suspected to be HIV infected. For HHV-8 serological testing, mothers gave written, informed consent for themselves and their children, and children ≥7 years old gave witnessed assent. The present study was approved by ethics boards at the National Cancer Institute and the Uganda National Institute for Science and Technology.

DNA was extracted from saliva and buffy coats by use of a QIAamp DNA extraction kit (Qiagen). Approximately 150 μg of DNA was used to detect EBV DNA by quantitative real-time polymerase chain reaction (PCR) on an ABI Prism 7700 machine (Applied Biosystems). Samples were tested in triplicate in an assay with primers specific for the EBNA1 gene, 5′-TCAT-CATCATCCGGGTCTCC-3′ and 5′-CCTACAGGGTGGAAAA-ATGGC-3′, and the labeled probe 5′-(FAM)CGCAGGCCCC-CTCCAGGTAGAA(TAMRA)-3′ [9]. EBV DNA levels were calculated as the average number of viral copies detected in triplicate wells for each sample; levels in saliva samples were calculated as the number of copies per milliliter of saliva, and levels in buffy coats were calculated as the number of copies per 1×10^6 peripheral white blood cells (PWBCs), on the basis of the copy number of the human endogenous retrovirus 3 gene (2 copies/cell) [10]. To minimize PCR contamination, dedicated rooms and equipment were used for reagent preparation, DNA extraction, and PCR amplification. Subjects with PCR results below the quantification limit were excluded from analyses of EBV DNA levels. Because the EBV DNA levels were skewed, they were $\log_{10}$ transformed before the analyses were conducted. The significance of associations between EBV DNA detection in either saliva or peripheral blood and sociodemographic variables were determined using contingency tables. The significance of associations between EBV DNA levels in either saliva or peripheral blood and sociodemographic variables, and P values for trend for variables with 3 or more categories, were determined using linear regression. The mean EBV DNA levels in saliva and peripheral blood cells of the children and mothers were compared using a 2-sample t test for independent populations. The odds ratios (ORs) and 95% confidence intervals (CIs) for detection of EBV in saliva and peripheral blood were estimated using logistic regression. Two-sided $P < .05$ was considered to be statistically significant.

RESULTS

The mean age of the children was 8.4 years (SD, 3.9 years). Nineteen children were <2 years old, including 1 child who was 11 months old (included in the 1–4-year-old group). The mean age of the mothers was 34.4 years (SD, 7.8 years). Among subjects with adequate samples, we detected EBV DNA in saliva from 90% (194/216) of the children and from 79% (175/222) of the mothers. In both groups, detection of EBV DNA was unrelated to sociodemographic variables (data not shown). Compared with their mothers, the children had higher levels of EBV DNA in saliva (median [interquartile range {IQR}], 5.2 [4.2–6.0] vs. 4.8 [3.7–5.6] $\log_{10}$ copies/mL of saliva; $P < .001$). For the children, levels of EBV DNA in saliva were unrelated to sex ($P = .57$) and age ($P = .66$), although they were weakly (albeit nonsignificantly) inversely related to maternal education ($P = .13$) and directly related to household density ($P = .10$) (figure 1). For the mothers, the median level of EBV DNA in saliva was higher in women 17–29 years old than in women ≥30 years old (5.1 vs. 4.5 $\log_{10}$ copies/mL; $P < .007$), was inversely related to education ($P = .03$), and was unrelated to household density ($P = .15$).

Among subjects with adequate samples, we detected EBV DNA in buffy coats from 86% (201/233) of the children and from 72% (166/230) of the mothers. As was observed for saliva, the frequency of detection of EBV DNA in buffy coats from both groups was unrelated to sociodemographic variables (data not shown). In contrast to the findings for saliva, levels of EBV DNA in buffy coats from the children did not differ from the levels in buffy coats from the mothers (median [IQR], 2.5 [2.2–2.9] vs. 2.7 [2.4–3.1] $\log_{10}$ copies/1×10^6 PWBCs; $P = .24$). For the children, levels of EBV DNA in buffy coats were unrelated to sex ($P = .18$), maternal education ($P = .16$), and household density ($P = .52$) but were strongly inversely related to age ($P = .001$) (figure 2). For the mothers, levels of EBV DNA in buffy coats were unrelated to age ($P = .77$), education ($P = .91$), and the other measures of socioeconomic status (figure 2 and data not shown).

EBV DNA was more frequently detected in buffy coats when detected in saliva, both for the children (OR, 3.1 [95% CI, 1.1–8.8]) and for the mothers (OR, 2.6 [95% CI, 1.3–5.1]). Because we previously found strong associations between water source and HHV-8 DNA shedding [8], we examined the associations between water source and levels of EBV DNA in saliva and buffy coats. Levels of EBV DNA were highest in buffy coats, but not in saliva, from children who consumed water from surface-water sources, were interme-
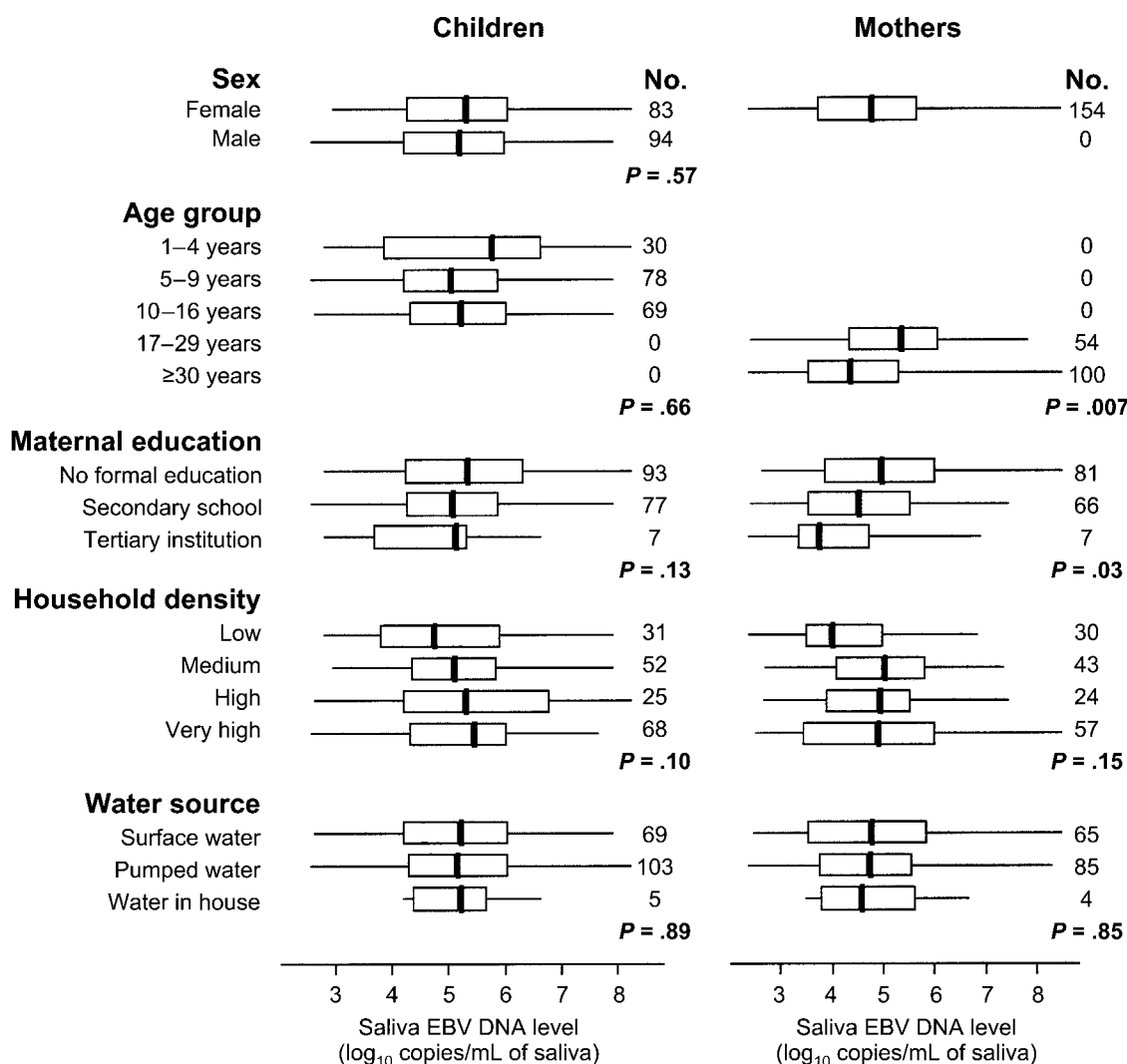
Figure 1. Comparison of levels of Epstein-Barr virus (EBV) DNA detected in saliva from children and their mothers, according to selected socio-demographic variables. The dark bars inside the boxes mark the medians, the left and right borders of the boxes mark the interquartile ranges, and the left and right extents of the lines outside the boxes mark the 10th and 90th percentiles, respectively. Also shown is the no. of subjects with quantitative EBV DNA measurements (excludes subjects qualitatively positive for EBV by polymerase chain reaction or with missing covariate data). Household density was calculated as the no. of persons in the house per the no. of rooms in the house (low, ≤ 1.50; medium, 1.51–2.24; high, 2.25–2.99; and very high, ≥ 3.00). P values for age, maternal education, household density, and water source are for trend.

diate in buffy coats from children who had access to water from a public tap or borehole, and were lowest in buffy coats from children who consumed water from private taps ($P = .001$; figure 2). Levels of EBV DNA in saliva and buffy coats from the mothers were not related to water source. Neither the frequency of detection nor the levels of EBV DNA in saliva or buffy coats from the children and mothers were related to residence location (i.e., urban, semiurban, or rural), use of mosquito bed nets, recent illness, or HHV-8 serostatus (data not shown).

Compared with HHV-8 DNA [8], EBV DNA was detected 5-fold more frequently in saliva (89% vs. 17% for the children and 86% vs. 17% for the mothers) and 8–14-fold more frequently in buffy coats (79% vs. 10% for the children and 72% vs. 5% for the mothers). When detected, levels of EBV DNA were, on average, 0.25 $\log_{10}$ copies higher in saliva and 0.5 $\log_{10}$ copies higher in buffy coats than the corresponding HHV-8 DNA levels ($P < .001$, for all; data not shown). In the children, EBV DNA was more frequently detected in saliva when HHV-8 DNA was detected than when it was not (100% vs. 88%; $P = .02$). However, detection of EBV DNA in buffy coats from the children was unrelated to detection of HHV-8 DNA (93% vs. 85%; $P = .20$). The corresponding EBV DNA detection frequencies for mothers with or without HHV-8 DNA
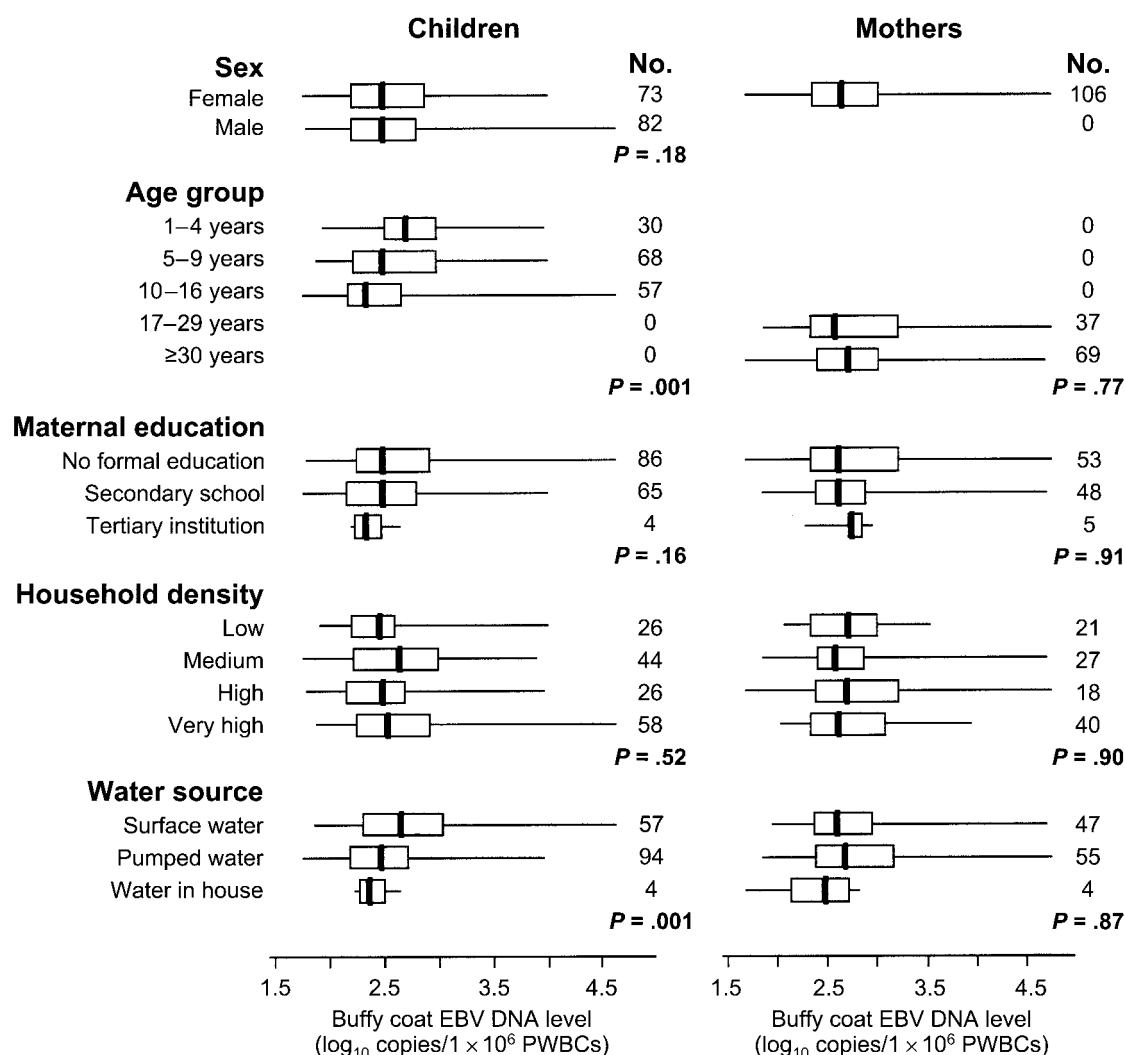
Figure 2. Comparison of levels of Epstein-Barr virus (EBV) DNA detected in buffy coats from children and their mothers, according to selected sociodemographic variables. See the legend for figure 1 for details. PWBCs, peripheral white blood cells.

were 91% versus 77% ($P = .12$) in saliva and 82% versus 72% ($P = .46$) in buffy coats.

DISCUSSION

This is the first study of EBV DNA in saliva and peripheral blood from asymptomatic subjects in Africa. EBV DNA was detected frequently and at high levels in both saliva and buffy coats from children with sickle cell disease and their mothers. Detection of EBV DNA in saliva correlated with detection in buffy coats, suggesting that viral levels in the oral compartment and in peripheral blood are closely correlated. The levels of EBV DNA detected in our subjects were high, indicating that EBV infection persists at high viral levels even in persons without apparent symptoms. The levels measured in the buffy coats from our subjects are similar to those reported in immuno-

suppressed transplant recipients in Western populations, which are typically 3.5–4.9 $\log_{10}$ copies/1 $\times$ 10^6 peripheral blood mononuclear cells [11].

EBV DNA levels in saliva and buffy coats were highest in the youngest age group (1–4 years). A recent study of asymptomatic children in Kenya also found very high EBV DNA levels in the peripheral blood of children 1–4 years old [6]. The high EBV DNA levels in these young children could be due to primary EBV infection; however, viral levels were still quite high in the older children and in the mothers in our study, yet these persons would have been infected many years earlier. Our results, and those from Kenya, suggest that the number of EBV-infected B cells is increased in EBV-infected persons in Africa. We speculate that recurrent malarial or other parasitic infections may be important. Malarial and other parasites affect the

immune system and may impair EBV-specific T cell immunity and, thus, influence EBV DNA levels in saliva or peripheral blood [12]. Alternatively, they may stimulate expansion of the pool of latently infected B cells [12]. In support of this hypothesis, EBV DNA levels in peripheral blood have been shown to be higher in children in high-intensity malaria-transmission areas than in children in low-intensity malaria-transmission areas in Kenya [6]. We cannot exclude or confirm a role for malarial or other parasites, because they were not assessed in the present study. However, ~17% of children in Kampala, Uganda, have asymptomatic smear-positive malaria, and 45% have PCR-detected malarial DNA [13]. Here, EBV DNA levels in saliva were associated with some (low maternal education and high household density), but not all, markers of low socioeconomic status. EBV DNA levels could be affected by socioeconomic factors that influence the age at infection, inoculum quantity, and the frequency of repeated exposures to EBV and/or other parasites.

In Africa, EBV is ubiquitous, whereas HHV-8 has a more restricted distribution. In the present cohort, EBV DNA was detected more frequently and at higher levels in saliva and buffy coats than was HHV-8 DNA. Although both viruses are γ-herpesviruses and are apparently transmitted via saliva, the higher EBV DNA levels in saliva may explain the higher transmissibility and younger age at infection for EBV than for HHV-8 [2, 14]. Our study suggests that infection with one virus is associated with increased viral shedding of the other. Genetic or environmental factors may be responsible for this correlation [15]. In contrast to these findings, HHV-8 DNA is detected infrequently and at barely detectable levels in saliva and buffy coats from persons in areas where endemicity is low, such as Europe and North America, except among men who have sex with men.

Our study is limited in that it analyzed children with sickle cell disease, who are prone to immunosuppression due to loss of splenic function from repeated infarctions. Thus, our results may not be generalizable to the whole population of Uganda. However, our findings are similar to those for apparently healthy Kenyan children [6]. They are also similar to the findings for the mothers of our children, who did not have sickle cell disease. These mothers must have been heterozygous for the sickle cell gene, but, as carriers, would not have suffered splenic infarction. In addition, our study was cross-sectional; thus, we could not determine longitudinal relationships between EBV DNA levels and clinical and sociodemographic factors. Third, we did not test for infection with malarial or other parasites, which could have influenced our results. Because our study is the first to evaluate EBV shedding in saliva in Africans, our findings need to be confirmed.

To conclude, EBV DNA was readily detected at high levels in saliva and buffy coats from relatively healthy subjects from Uganda. Because of its association with Burkitt lymphoma, a malignancy that is endemic in Africa, it will be important to understand the factors that control EBV infection.

References

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